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MOULDS AND BACTERIA ON EGG COLLECTIONS.

BY FREDERIC H. KENNARD.

DURING the summer of 1916 there came into my possession a small collection of New England eggs, some 1250 in number, which I particularly prized, as having been personally taken by a friend who had passed away sometime before, but with whom I had been on many a collecting trip; and with the history of whose personally collected eggs I was rather intimately acquainted.

Upon acquiring the collection I was surprised to discover upon a number of the eggs, small spots of what appeared to be a tawny mould scattered over the surface. These spots, varying in color from "tawny-olive" to the brighter "ochraceous-tawny" of Ridgway¹ were small, often so minute as to require a glass for their discovery; and seemed to be pretty generally distributed over the surface. Many of the infected eggs had but a spot or two, which were hard to distinguish in some cases from markings, while on others there were larger and very evident spots, often with a characteristic dendritic appearance, and many of them with fine filaments of the cotton on which they had been laid still adhering. On a very few of the eggs, soiled seabirds' and the like, there seemed to be other mould of some common kind.

I applied to several friends at this time for advice; but only two of them had ever encountered similar trouble, and while they knew of no sure cure, I was advised to clean the eggs with Bon Ami soap, applied with warm water and a soft rag; and then after drying, to put them away in a dry place.

Upon a careful examination of every egg in the collection with a ten-power magnifying glass, I found that apparently one or more eggs in eighty-nine sets, out of a total of 262,—over thirty per cent,—were more or less infected.

Of these I broke up thirteen sets—several accidentally, and the rest deliberately,—in order to find out whether or not the insides of the shells were similarly affected. Aside from a very few exceptional cases, where the growth happened to develop about the

¹ Color Standards and Color Nomenclature by Robert Ridgway.

blow-hole, and for perhaps half a millimeter around its inner edge the insides of the eggs appeared to be free of the trouble.

After this examination, I carefully cleaned all the eggs, the infected, as well as those which appeared to be uninfected, with Bon Ami soap;¹ and found the spots readily removable, except in a very few aggravated cases, in which the tawny growth seemed to have eaten into the substance of the shell, and left a slightly discolored pock-mark. The markings of the eggs seemed to be pretty well fixed, with the exception of the Falconidae—Duck Hawk, Pigeon Hawk and Sparrow Hawk—and some of the more highly pigmented seabirds, —Murres, Razor-billed Auk and the like, on which great care had to be used not to rub off the color.

After cleaning the eggs, I washed each of them with grain alcohol, in the hope that this might kill any of the growth or spores that might remain, and then after thorough drying, put them away in airtight cases, the apparently uninfected eggs in one, for further observation; and the infected eggs in another; and kept them all away from my own collection, which had always been spotless.

Having necessarily by this time become very much interested in the problem, I sent a few specimens during the autumn of 1916 to the Bureau of Biological Survey, Washington, D. C.; and was informed by them, after they had submitted the specimens to experts in the Bureau of Plant Industry, that the growth was a fungus, a species of penicillium "grown on albumen left on the surface of the eggs at the time they were blown," and that the color of the spots was probably due to dirt having accumulated at those points. It was thought that all the eggs needed was a thorough cleaning, and it was wisely suggested that I scrub the eggs with a solution of mercuric chloride, commonly known as corrosive sublimate, 1 to 500, dry thoroughly, and then keep them in a dry place, as "unless the egg is kept in a very damp place, there appears to be no danger of continued growth of the fungus."

Now I am no cryptogamist, and there doubtless were spots of penicillium growing on some kind of organic matter on those parti-

¹ I can recommend Bon Ami soap for anything of this sort; mould, dirt or even ink spots being, in most cases, readily removed by it.

cular eggs which I sent the Biological Survey—they were a few my friend had acquired by exchange, Seabirds, Murres, Cormorants, etc. and we all know what they may look like,—but inasmuch as my friend who had made the collection had always taken scrupulous care in blowing, cleaning, and rinsing his personally taken specimens, which were apparently just as badly infected as the Murres' and Cormorants' eggs; and had kept them in a specially built and practically dust-proof cabinet, in a dry place, it did not seem to me that the penicillum could be the cause of all the trouble. The coloring of the tawny spots seemed not to be due to dirt, and the spots themselves were too evenly distributed, not centered about the blow-holes, and on shells that appeared perfectly clean. They had also flourished in too dry an atmosphere to fit the diagnosis.

Illness prevented my treating the collection with mercuric chloride at this time; but as I had cleaned the eggs pretty thoroughly, and washed them with grain alcohol, and put them in a dry-, place as advised, I hoped that perhaps no further treatment might be necessary.

A part of my collection is kept on the best quality absorbent cotton in pasteboard trays, in drawers in airtight metal cases; while those sets that were collected with their nests, are kept in specially made glass-topped pasteboard boxes, airtight, bug, fungus and fool-proof; and all in the third story in my house, dry, warm and well ventilated at all seasons; and I had previously never had any trouble of any sort.

During the ensuing months I inspected the eggs from time to time; and after a year or so found, to my disgust, that minute spots of the tawny growth were reappearing; but it was not until the autumn of 1918 that I was able to examine the collection again carefully with a glass. I then found that the growth, though apparently very slow of development, had started up again on about thirty per cent of the previously infected sets; and what was worse, had begun to show itself also on about twenty per cent of those that I had thought were uninfected; and this on specimens that had all been cleaned, as well as it was mechanically possible, of all albumen, dirt, or whatever else the growth was supposed to live upon. Evidently I had locked into these cases, in the cotton or

elsewhere, and in spite of all the pains I had taken, enough spores to perpetuate the trouble.

I cleaned them all again with Bon Ami soap, having again to remark a large number of the specimens; and then gave them all a bath, as prescribed by the Biological Survey, in a solution of mercuric chloride, one to five hundred; submerging them for from ten to twenty minutes according to the size of the eggs and the toughness of their shells; and then after rinsing them in fresh water, submerged them in another bath of fresh water for another period of from ten to twenty minutes, in order to be sure that none of the solution might remain inside, in such strength as to cause discoloration later about the blow-holes, which I was told might occur on improperly washed eggs when exposed to the light. As a matter of fact out of 1250 eggs, only one later showed a slight discoloration.

As I have never found the trouble on the inside of the shell, except in a very few exceptional cases immediately around the inner edge of the blow-holes, I figured that if the eggs were totally submerged, holes down, the solution would be sure to press up into the blow-holes far enough to reach all the trouble.

I used a series of enamel iron pans, deep enough to submerge the largest eggs, a number at a time, without overflowing; and I kept the eggs submerged by means of floating wooden covers, fitted to the pans, and weighted down sufficiently with small blocks of wood, so as to keep the eggs properly beneath the surface. I handled the eggs in the solution with surgeon's rubber gloves, and as was to be expected, lost a few of the more delicate specimens. A Warbler's egg, that has been taken in an advanced stage of incubation, does not take kindly to such heroic treatment, even in water; and too long continued a bath seemed to weaken a few of them perceptibly.

After blowing all of them that contained any appreciable amount of the water in which they had last been rinsed, I placed them, holes down, on clean absorbent cotton for twenty-four hours; and then placed them, holes up, in trays in an open cabinet, to the front of which I applied an electric heater for another twenty-four hours, to insure their being absolutely dry. I then placed them on clean cotton,—the best sterilized absorbent,—in clean trays, in clean

drawers, previously washed with mercuric chloride, in similarly treated cases; and though I have watched them carefully to date—August 12, 1920—have been unable to discover any recurrence of the trouble.

In order to find out how widespread the trouble might be, and under just what conditions it was most apt to occur, I communicated either personally or by letter, with a number of ornithologists throughout the United States and Canada. Of these, sixty-four either owned or had charge of egg collections, and twenty-six or over forty per cent, had had more or less experience with foreign growths of some sort on their eggs, while thirteen of these, or about twenty per cent, had had their collections seriously affected. Four had acquired collections with infected eggs, but had gotten rid of the trouble with comparative ease; and nine had only had isolated cases from which the trouble never seemed to spread. Personally, I believe, that if examined carefully, a majority of the collections would have been found to have contained a few infected eggs. The tawny growth seems to have been the cause of most of the trouble, as only a few had had much experience with commoner forms of mould.

One of the best known of our oölogists writes me that “the mould or fungus you refer to is, aside from the dermestes, the only thing the oölogist has to contend with, except light and dampness,” and an ornithologist of international reputation writes me “as regarding the fungus that attacks eggs, I can only say that my collection is being destroyed by it, and I do not know how to stop it.”

I saw a collection the other day, and I know of a number similarly affected, which had been stored in an airtight “Cambridge can” in a friend’s warm, dry attic for a number of years. It was a collection that had been made and kept with a great deal of care, but which had become infected in some way with the tawny growth, and absolutely ruined by it.

With regard to the conditions under which the various collections were kept, I find that of the twenty-six infected collections, the thirteen in which the trouble never became serious were all kept in warm, dry, and comparatively well ventilated rooms; while of the thirteen that were seriously infected, a majority were kept under questionable conditions, in rooms that were cold and damp

and ill-ventilated, at least during certain seasons of the year, and four were kept under supposedly favorable conditions. Yet two of these last, kept in airtight metal cases, in warm, dry attics, were the worst infected collections that came to my notice. It may be only a coincidence, but in a number of collections the growth seemed to have spread also to the cotton on which the eggs were placed, and each time on cotton of inferior quality.

Of the thirty-six collections that were reported uninfected, twenty were kept in rooms that were apparently well aired and dry, three were kept in rooms that were damp, unheated, or closed, at least a part of the year; while thirteen of my correspondents failed to describe the conditions under which their collections were kept.

Most of my correspondents thought the cause of the trouble was dampness, particularly at certain seasons of the year; a number added "dirt;" and two or three laid all the trouble to the "small hole crank" who does not blow or rinse his eggs properly; while nearly all those that had been free from the trouble, were quite sure that their particular method of keeping collections was responsible for that freedom; and one or two enthusiasts were equally sure that they owed that freedom to the salubrious climate in which they lived. As a matter of fact, I found that the growth occurred there, just as elsewhere, and may apparently occur anywhere.

Among the numerous remedies suggested were, cleaning with warm water, ordinary soap, Bon Ami soap, Sapolio, heat, moth-balls, fumigation with bisulphide of carbon or with formaldehyde candles, or with dishes of formaline in each cabinet; immersion in salt water or gasoline; poisoning the interior of the egg with a weak solution of copper sulphate; treating the eggs with solutions of formaldehyde, peroxide of iron, bicarbonate of soda, carbolic acid, acetone, mercuric chloride, and izal. It was also recommended that unslaked lime or calcium chloride be kept in dishes in cabinets to absorb undue moisture; and one of our foremost scientists, while not believing in moth-balls as a remedy, was inclined to believe that they might possibly act as a deterrent.

Of the specimens that seem most apt to be attacked, one well known collector writes "smooth, plain eggs, notably duck eggs,

are affected, but also white eggs, such as owls, wood-peckers, kingfishers."

Another writes that "the Raptores are the favorite eggs attacked;" while still another writes "the glossy eggs are seldom affected unless very dirty; soft-shelled eggs with roughly or finely granulated surfaces are most seriously affected, e. g. my Gadwalls, 'Phoebes' and Swallows' eggs show the highest percentage."

In my own case the growth seemed to be pretty generally distributed, but the eggs of grouse and gallinules were worst affected, while the Raptores were practically free. As a matter of fact, I find, after looking over a number of infected collections, that the growth seems entirely impartial in its tastes, and those eggs which happen to be within reach of the point of infection, may become infected, apparently regardless of color or texture.

After duly assembling, tabulating, and endeavoring to digest all the above seemingly rather contradictory data, in the spring of 1919, feeling quite sure I had permanently cured the trouble in my collection, and being more curious than ever as to the cause thereof, I asked Prof. S. C. Prescott of the Massachusetts Institute of Technology to investigate the cause of the trouble; and to suggest, if he could, some better method of getting rid of it. That which I had adopted was clumsy and bothersome and was hard on the more fragile eggs, because of the repeated handling and necessary re-marking, and I wanted, if possible, to discover some method of fumigating, as with carbon bisulphide, formaldehyde or something of the sort, which would be convenient for all collectors.

While Prof. Prescott was not able to give all the time and attention to the problem he would have liked, his investigations were carried on for a number of months, during which a large number of eggs were examined, from a number of infected collections, and from which there were obtained numerous cultures for a long series of tests.

Under date of July 31, 1920 Prof. Prescott writes as follows:

"Repeated examination of the infected birds' eggs has shown that the discoloration appears to be due to a large spore-forming bacillus which develops brownish colonies very slowly on the external surface. It is possibly an undescribed species, although cultures and microscopic appearances are so close to the potato bacillus that there can be no doubt

as to the group to which it belongs, and I have classified it provisionally as a variant of the *Bacillus mesentericus fuscus*. Occasional eggs show also the presence of fungi, but the main cause of the trouble appears to be the bacillus. This organism grows even in rooms which are very low in humidity, and may therefore appear in what would be dry chambers. While treatment with corrosive sublimate or other strong disinfectant-solutions is troublesome and time-consuming, and the ideal method of disinfection of large collections would be by use of gaseous disinfectants such as formaldehyde, the high resistance of the spores makes the fumigation of the eggs by formaldehyde or other gaseous disinfectants uncertain, as the destruction may not be complete and successful. If spores are not destroyed, they may possibly develop later with slight changes in the atmospheric conditions. We are, therefore, at this time unable to recommend any easier procedure than treatment with solutions of corrosive sublimate which appear to be effective. A treatment of five or ten minutes with a 1 to 1000 solution will probably be sufficient to destroy the infection and prevent further developments."

To summarize, it appears from the evidence obtained, that specimens should not only be thoroughly blown, rinsed, and cleansed of all organic matter when added to one's collection; but should be kept in dry, well ventilated rooms in dust-proof cases. Under such conditions, ordinary moulds, "mucor, aspergillus, penicillium, eurotium, and a few less common forms," all of which were found and isolated by Prof. Prescott, are little to be feared, and are controlled with comparative ease.

On the other hand, the tawny bacillus mesentericus fuscus, though slow of growth, seems when once introduced, to spread under just such conditions; to grow upon the surface of the shell itself, which it may injure; and to be particularly difficult to eliminate; confinement in airtight cases in a dry place seeming at times to aid in its development.

To recapitulate regarding treatment; upon discovery of the trouble, all sets of infected eggs, and when possible, all those that have been exposed to infection, should be cleaned, when necessary, with Bon Ami soap, applied with an old linen handkerchief, or soft rag of some sort. Particular pains should be taken at this time to re-mark with great care, all eggs from which the set-number has been erased during cleaning, and it is right here that the casualties are most apt to occur.

Then the eggs should be totally submerged, blow-holes down,

in a solution of mercuric chloride, 1 to 500. Get the ordinary commercial white tablets from your druggist, and dissolve 4 to the quart of water, in glass or enamel iron pans or dishes of some sort, not susceptible to the action of mercuric chloride, and deep enough to submerge the largest of the infected eggs.

Since treating the collection, we have found by further experimenting with cultures in the laboratory, that a submergence of one and a half minutes in mercuric chloride, 1 to 500 will kill the spore-bearing bacteria. Under the less favorable home conditions, it seems to me that a submergence of from three to five minutes, according to size and texture of the egg should not only be ample time to kill all bacteria, but can do no harm to the shell itself.

After this bath, the eggs should be rinsed in running, or frequently changed water, and then again submerged, in a bath of fresh water, for a period of from 5 to 10 minutes according to the size and texture of the eggs.

When there are a number of eggs to be treated, surgeon's rubber gloves may be used, and several sets of pans, in order to facilitate handling, as well as to insure against mixing the eggs. I never place two sets of the same kind, or even similar eggs, in the same bath at the same time.

After this bath, blow out any water that may remain in the eggs, then wipe carefully, and place, blowholes down, on a sheet of absorbent cotton for 24 hours or until drained; then turn, and place holes up for another 24 hours, in a warm dry room, and if possible, help the drying-out process with a little extra gentle heat, as for instance, an electric reflector or something of the sort.

When the eggs are put away in the cabinet, particular care should be used to see that whatever material they are placed on, as well as the trays or drawers they may be placed in, are absolutely clean, and free from all possible previous contamination by the fungi or bacteria. A few spores inadvertently left on the cotton or sawdust, or whatever medium is used, may bring on an exceedingly unwelcome recurrence of the trouble.

I got rid of it with infinite pains, as described above; and while I realize that my method was far from ideal, it certainly was effective, even though Prof. Prescott seems to think the solution

stronger than necessary; and as such I offer it to my fellow-sufferers, until some equally sure but more convenient method may be devised.

With at least two out of every five collections more or less infected, and at least one out of every five seriously affected by the trouble, and with specimens constantly being exchanged between collectors, the situation seems to demand attention.

After this all eggs received by me from doubtful sources will be quarantined and treated with corrosive sublimate. Eternal vigilance should be the price paid by those that do much exchanging; and unless some better method of treating the infected collection is devised, those collectors who really value their eggs, should keep their sets either in tight glass topped drawers, or better still in individual, spore-proof, glass topped paste board boxes¹, where the accidental introduction of an infected set can do little harm. The private collections of the late William Brewster, Messrs. John E. Thayer, John Lewis Childs, and a number of others that I know of, are kept in this way. It seems to me the only way for museum collections, where eggs generally fail to get the loving care they do from individuals; and the sooner the rest of us adopt this method, the sooner we may be able to control or check the spread of the disease. It is the ideal way of keeping eggs; and while it may cost a little more money, it is certainly worth while. When one thinks of all the money one spends on collecting trips, and in the general rounding up of specimens, the extra cost of such boxes is hardly worth considering.

Some three months after the above paper had been sent to the Editor, I chanced, while attending the meeting of the A. O. U. at Washington, D. C., to foregather with Mr. J. H. Riley of the U. S. National Museum, and from him learned of a method of cleaning eggs, which had been used successfully at the Museum sometime prior to 1903, when Mr. Riley had treated a number of infected eggs, upon which there seems to have been no recurrence of the trouble.

¹ Beautiful boxes of this sort are made to order in sizes desired, by the Dennison Manufacturing Co., 26 Franklin St., Boston, Mass.

Chlorinated soda and "Javelle Water," which is chlorinated potash, seem to have been used impartially and with equal success. Both are strong bleaching agents commonly used as disinfectants, in the removal of mildew, etc., and in museums for the cleaning and bleaching of bones. The use of these chemicals for the cleaning of eggs, was confided to Mr. Riley by Dr. W. L. Ralph, who succeeded Major Bendire as custodian of birds eggs at the U. S. National Museum. Mr. Riley writes that "Dr. Ralph always maintained that the use of this solution on eggs was his secret, and would never let me tell of its use, but as he is dead now [he died in 1907] you can make any use of the knowledge of this preparation you have a mind to. In fact I think its use should be more generally known."

With regard to method of treatment, Mr. Riley writes as follows: "Pour some of the liquid into a shallow china saucer, or other earthen vessel, then if eggs are to be cleaned, dampen an old linen handkerchief, and carefully wipe the spot to be cleaned, and note the action. When the stain has been removed, thoroughly wash the egg in clean water, and allow to dry. Care should be taken with pigmented eggs, but white eggs need not be watched so closely."

. . . "I should not think of trying to clean pigmented eggs without the greatest care, and as follows: Barely have the cloth damp with the solution, and have a basin of clear water right at hand. Then lightly touch the spot to be cleaned with the damp cloth, and lightly brush it. On the first sign of bleaching, or when the stain is removed, thoroughly wash in clean water."

. . . . "Eggs so badly nest stained that the markings could not be seen at all, I have cleaned so nicely that you would never know that they had been stained at all."

Mr. Riley writes further that "This solution can also be used in removing embryos from badly incubated eggs. Introduce it into the egg with a syringe. I have left it overnight in a large egg. You will have to experiment to find out how long to leave it in an egg, as it varies with condition and size of egg, of course."

Prof. Prescott and I have since done considerable experimenting, with both the Chlorinated soda and Javelle Water; and while they certainly proved to do all that Mr. Riley said they would, I should hesitate to recommend them for general use, at least so far as the removing of spore-bearing bacteria is concerned.

Both chemicals act very quickly upon the shell itself, unless diluted with four parts water, and even then should be used with the greatest care, especially on what may be called surface-pigmented eggs. In addition to the risk attached to this method of treatment, the mere wiping of eggs can hardly be as thorough as a total submergence, (it is difficult for instance to wipe around the inside of the blow-hole); and as we find that, under favorable laboratory conditions, it takes chlorinated soda, one to four parts water even longer to kill the spore-bearing bacteria than it does corrosive sublimate, a submergence of pigmented eggs long enough to be affective is apt to injure both texture and markings. Bon Ami soap and warm water will be found, in most cases, to be just as affective, so far as the actual cleaning of the shell is concerned, and far less dangerous.

While we had no eggs with embryos, on which to experiment, the use of these chemicals in softening up membranes and other matter that might remain inside the shells, was truly remarkable. A solution of one part chlorinated soda and four parts water, shaken up in an egg, would quickly soften any remaining membrane; but small eggs left over night showed a distinct deterioration, the chemical having eaten its way through, and leaked out of some of the smaller ones, while eggs even as large as Spotted Sandpiper's became very fragile.

If I had a few large unspotted eggs that needed cleaning I might try Dr. Ralph's method, but in any other case, it seems to me that treatment with corrosive sublimate is, in comparison, not only safer, but better in every way.

Thanks are due to the many ornithologists, oölogists, members of the Biological Survey, Mr. Riley and others, and to Professor Prescott in particular, who have, with considerable patience helped me in this investigation.

Dudley Road, Newton Centre, Mass.